Journal of Ethnopharmacology 131 (2010) 443–450

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice

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ARTICLE INFO

Article history:
Received 3 February 2010
Received in revised form 27 May 2010
Accepted 6 July 2010
Available online 13 July 2010

Keywords:
Rosmarinus officinalis
Diabetes mellitus
Wound healing
Excision wound
Histological analysis

ABSTRACT

Aim of the study: Rosmarinus officinalis (Rosemary) used in Jordanian folk medicine for wound management and treatment. Therefore, the present study was conducted to assess the healing efficacy of both aqueous extract and essential oil of the aerial parts on alloxan-induced diabetic BALB/c mice.
Materials and methods: Two full-thickness round wounds were created in the dorsal area of each mouse. Animals were divided into four groups of twenty mice each: untreated normal, untreated diabetic, aqueous extract- (intraperitoneal injection of 0.2 ml at a dose of 10% for 3 days) and essential oil-treated diabetic mice (topical application of 25 μl/excision wound, twice a day for 3 days). For 15 days, the wounds were visually observed; blood glucose level, body weight, regenerated granulation tissue weight and the percentage of wound contraction were measured. On days 6 and 15 after wounding, the animals were sacrificed and the histology of wound area was examined.
Results: Significant positive differences ($p < 0.01$) between treated and control groups were observed at different aspects of diabetic wound healing process. Reduced inflammation and enhanced wound contraction, re-epithelialization, regeneration of granulation tissue, angiogenesis and collagen deposition were detected in the treated wounds.
Conclusions: Results indicated that the essential oil of *Rosmarinus officinalis* was the most active in healing diabetic wounds and provide a scientific evidence for the traditional use of this herb in wound treatment. However, further scientific verification is required to confirm and assess the range of wound healing potential of essential oils of Rosemary chemotypes.

1. Introduction

The use of medicinal plants and herbs has recently been increased throughout the world for the maintenance and improvement of health and for the treatment of various human conditions and diseases. About 60% of the world population and 60–90% of the population of developing countries rely on traditional medicine for their primary health care (Kunwar and Bussmann, 2008). In addition to approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1–3% of synthetic modern drugs (Mantle et al., 2001). These present a clear indication for the crucial role of medicinal plants as therapeutic alternatives to conventional medicine.

Based on ethnobotanical surveys, several species of plants and herbs with healing potential of wounds/burns are widely distributed in Jordan and Palestine and extensively used by the inhabitants of the area and traditional healers (Al-Khalil, 1995; Oran and Al-Eisawi, 1998; Ali-Shtayeh et al., 2000). However, few of them have been investigated to confirm their potential healing effect in Jordan (Abu-Al-Basal, 2001; Khalil et al., 2007).

Herbal medicines in wound management involve disinfection, debridement and the provision of suitable environment for natural healing process (Shanmuga Priya et al., 2002). In fact, alternative medicine are of less toxicity and with fewer side effects compared with conventional medicine, and hence it is important to introduce a scientific validation for the medicinal effect of plants used in traditional medicine.

Rosmarinus officinalis L. (Family Lamiaceae), popularly named rosemary, is a perennial herb with fragrant evergreen linear leaves used as a spice and a flavouring agent in food processing (al-Sereiti et al., 1999). Rosemary is grown in many parts of the world, including Jordan, where is mostly cultivated or grown naturally and is named by the local inhabitants with Hasalban or Iklil al-Jabal (Karim and Quraan, 1986; Al-Khalil, 1995; Al-Qura'n, 2009). The aerial parts of the herb has a long tradition of use as an antispasmodic in renal colic and dysmenorrhoea, anti-rheumatic, anti-aging (al-Sereiti et al., 1999; Abu-Rabia, 2005; Al-Qura'n, 2009), in relieving respiratory and digestive disorders, hypertension, kidney stones, sugars in blood (Everest and Ozturk, 2005; Lev, 2006), in stimulating circulation and nervous system and in treat-

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doi:10.1016/j.jep.2010.07.007

ing skin diseases such as hair loss, infections and healing of wounds (Abu-rmaileh and Afifi, 2000; Pieroni et al., 2004; Heinrich et al., 2006).

Healing of wounds is considered necessary for the removal of damaged tissues and/or invaded pathogens from the body as well as to restore the continuity and architecture of a cutaneous or visceral defect (Matsuda et al., 1998; Lerman et al., 2003). Cutaneous wound healing is a complex process that involves coordinated interaction between a number of cell types, extracellular matrix (ECM) molecules, and growth factors in three overlapping phases: coagulation and inflammation, proliferation and remodeling (Bohl Masters et al., 2002; Eming et al., 2007).

Diabetes mellitus constitutes one of the most important public health problems due to its high prevalence and enormous social and economic consequences (Silva et al., 2007). Delayed cutaneous wound healing is a chronic complication in diabetic patients and is caused primarily by hyperglycemia, diminished expression of cytokines, oxidative stress, vascular insufficiency and microbial infections (Lerman et al., 2003; Sivan-Loukianova et al., 2003; Hirsch et al., 2008; Abu-Al-Basal, 2009). Several other diabetic complications like neuropathy, nephropathy, atherosclerosis and foot deformities contribute to the severity of the disease and in the development of chronic wounds in diabetic patients, that might be complicated leading to ulceration, necrosis and amputation (Lerman et al., 2003; Hirsch et al., 2008). Diabetes mellitus is life-threatening and becomes the third largest killer of humans after cancer and cardiovascular diseases, even with the use of several synthetic recent drugs for effective treatment options (Li et al., 2004).

Healing impairment of diabetic patients is still a serious clinical problem for physicians worldwide due to unclear etiology; therefore a new era in wound healing research is required involving new treatment strategies to deal with this emerging issue. One of these is the use of medicinal plants as a source for natural products to explore new therapeutic agents/tools intended for diabetic wounds management and treatment.

The potent antioxidant and ethnopharmacological properties of *Rosmarinus officinalis* make it an excellent source for searching a new medicinal target as wound healing, since currently there are no studies for the influence of such herb on healing chronic wounds or extensive burns. Therefore, the present study has been carried out to assess the efficacy of the ethnobotanical use of *Rosmarinus officinalis* on various parameters and stages of cutaneous wound healing process in diabetic BALB/c mice.

2. Materials and methods

2.1. Plant collection

Rosmarinus officinalis herb was collected in April, 2008 from Al-al-Bayt University campus, Mafraq, Jordan. Taxonomic identity of the herb was confirmed at the Herbarium of the Department of Biological Sciences, University of Jordan, Amman, Jordan. A voucher specimen (No. RMJD-4-008) has been deposited in the Department of Biological Sciences, Faculty of Science, Al-al-Bayt University, Mafraq, Jordan for future reference.

2.2. Plant extract preparation

Essential oil was extracted from 500 g of fresh and cleaned aerial parts of *Rosmarinus officinalis* by steam distillation method. In which, water was heated to produce steam that pass on the herb material to carry the most volatile chemicals. Following by cooling and condensation of the vapor mixture and the resulting distillate was collected and separated from the layer of water by extraction. For aqueous extract preparation, fresh and cleaned aerial parts of the herb (100 g) were grounded into solution with 300 ml of distilled water using an electric blender. After centrifugation of the mixture for 10 min at 4100 rpm, the supernatant was collected and filtered through Whatman filter paper no. 2 under vacuum. Both essential oil and aqueous extract were separately kept in closed vessels at 4 °C in a refrigerator for further use.

2.3. Animals

Male BALB/c mice 6 weeks of age (18–20 g) were obtained from the animal house of the Department of Biological Sciences, Yarmouk University, Irbid, Jordan. Mice were kept under specific pathogenic-free conditions, housed, fed and treated in accordance with the international guidelines principles of laboratory animal use and care (Hedrich and Bullock, 2006). They were maintained on standard pellet diet and water *ad libitum* for 2 weeks to be acclimatized prior to the investigation.

2.4. Diabetes induction

Mice were weighed and their fasting blood glucose was determined before inducing diabetes. After fasting for 18 h, mice were intraperitoneally injected with 0.2 ml of alloxan monohydrate (Sigma–Aldrich Canada, Oakville, Ontario, Canada), freshly dissolved in 0.90% saline at a dose of 100 mg/kg body weight for 3 consecutive days. Normal control mice only received physiological saline in the same way. Symptoms of diabetic mellitus appeared on mice, within 1 week after the third injection of alloxan. Hyperglycemia was confirmed by measuring fasting blood serum glucose levels of the mice through three consecutive determinations. Mice with a blood glucose level above 16.7 mM were considered successful diabetic model of hyperglycemia and used for experiments as recommended previously (Zhang et al., 2009).

2.5. Wounding

After 24 h of hyperglycemia confirmation, mice were anaesthetized with 350 g/kg body weight of chloral hydrate (Scharlu chemie, S.A., Barcelona, Spain). Full-thickness excision skin wounds were performed as described previously (Gutiérrez-Fernández et al., 2007). Briefly, hairs on the dorsum of diabetic and normal non-diabetic mice were shaved, the exposed skin area were cleaned with 70% ethanol, and two full-thickness round skin wounds (4 mm diameter) were created on the same mouse along the dorsal middle line using sterile biopsy punch equipment (Revolving punch pliers, Germany). The two wounds were separated from each other by at least 1 cm of unwounded skin. The wounds were left open without any dressing material for the duration of the study. Wound healing was monitored by taking photographs on 0, 3, 6, 10, and 15 days after wounding.

2.6. Mice grouping and treatment

After wound creation, diabetic mice were randomly divided into three groups of twenty each, according to similar body weights, besides non-diabetic normal group. Various doses of plant extract and/or essential oil were preliminary tested for their tolerance in BALB/c mice to select the optimum dose intended for treatment of experimental animals. Group I: non-diabetic mice left without treatment as normal control. Group II: diabetic control mice treated with vehicle by intraperitoneal injection of 0.2 ml distilled water. Group III: diabetic mice treated with aqueous extract by intraperitoneal injection of 0.2 ml at a dose of 10% (v/v) once a day for 3 consecutive days. Group IV: diabetic mice treated with pure essential oil by topical application of 25 μl per each excision

Table 1
Effect of *Rosmarinus officinalis* aqueous extract and essential oil on blood glucose level in alloxan-diabetic mice for 15 days after cutaneous full-thickness excision wound creation.

Group	Day after wounding				
	0	3	6	10	15
	Blood glucose level (mM)				
I	05.82 ± 0.34	05.93 ± 0.27	06.25 ± 0.29	06.18 ± 0.15	05.95 ± 0.13
II	22.36 ± 1.00*	22.33 ± 0.87*	23.13 ± 0.28*	25.47 ± 0.28*	22.33 ± 0.32*
III	22.13 ± 1.28*	22.57 ± 1.04*	20.88 ± 0.29*	15.82 ± 0.57**	12.87 ± 0.38**
IV	23.48 ± 1.03*	20.53 ± 0.61*	15.98 ± 0.30**	12.23 ± 0.42**	10.67 ± 0.54**

Data are expressed as means ± SEM for six mice in each group. I: non-diabetic control; II: diabetic control; III: aqueous extract-treated diabetic group (I.P. injection of 0.2 ml at a dose of 10% for 3 days); IV: essential oil-treated diabetic group (T.A. of 25 μl/excision wound, twice a day for 3 days).
* Statistically significant when compared to non-diabetic control group (I) at $p < 0.01$.
** Statistically significant when compared to diabetic control (II) at $p < 0.01$.

wound of the mouse twice a day for 3 days. Mice were individually housed, maintained on normal food and water *ad libitum*, and those which showed infection signs were separated and excluded from the study.

2.7. Body weight and serum glucose measurement

Body weight and serum glucose level were determined immediately before wounding and after days 3, 6, 10, and 15. Blood samples (100–200 μl) were collected from anaesthetized experimental mice and controls via tail vein. Blood serum was prepared according to the method of Tsuneki et al. (2004). The samples were kept on ice for 1 h and then centrifuged at 16,000 × *g* for 2 min using micro centrifuge (TM 1-14, Sigma, Germany). The collected serum was immediately analyzed for glucose levels by glucose oxidase/peroxidase method using glucose kit (Linear Chemicals, S.L., Barcelona, Spain). Serum glucose levels were expressed in mM.

2.8. Wound contraction

Mice were photographed at the time of wounding (0 day) before treatment with the extract or essential oil and again on days 3, 6, 10 and 15 after wounding. The wound surface area was measured from the traced outline of a digital image of the wound by planimetry as described by Flanagan (2003). The percentage of wound contraction was calculated using: % wound contraction = $(A_0 - A_t)/A_0 \times 100$. Where A_0 is the original wound area and A_t is the area of wound at specific time period after wounding (Yates et al., 2007).

2.9. Granulation tissue

The granulation tissue of mice ($n = 3$/group) that was formed on the excision wounds, on days 3, 6, 10, and 15, was excised and the wet weight was recorded.

2.10. Histology

Two mice from each group were sacrificed 6 and 15 days after wounding. Biopsy (3 mm) from each wound site was excised to a depth sufficient to include the newly formed granulation tissue, adjacent wound edge and underlying muscle surrounded by a margin of normal skin. Tissue biopsies were fixed in 10% formalin, processed in the routine way for histological evaluation and then embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin for general wound analysis and additional sections 15 days after wounding were stained specifically with Masson trichrome for the assessment of collagen content and maturation within the dermis.

2.11. Statistical analysis

Results are expressed as means ± SEM (Standard Error of the Mean). Comparisons between groups were performed using paired Student's *t*-test on a statistical software package (SPSS). Differences were considered significant, if *p* value is less than 0.01.

3. Results

3.1. Blood glucose level

Significant increase in blood glucose level was observed in alloxan-induced diabetic mice compared to non-diabetic normal mice during the entire experimental period (Table 1). Both aqueous extract and essential oil exerted significant decrease in blood glucose level on days 10 and 15 after wounding when compared to the untreated diabetic group. However, the hypoglycemic effect of essential oil was more significant than that observed with the extract on day 6 after wounding.

3.2. Body weight

Body weight has decreased significantly in alloxan-induced diabetic mice with respect to normal non-diabetic control for the duration of the entire experiment. Treatment with either aqueous extract or essential oil significantly increases body weight toward the non-diabetic normal level, specifically on days 10 and 15 after wounding. However, essential oil exerted a more significant effect than the aqueous extract in improving body weight gain by diabetic mice (Table 2).

3.3. Granulation tissue

There was significant difference in the granulation tissue wet weight between untreated diabetic and non-diabetic normal groups at various days of wounding (3, 6 10 and 15). On day 3, the wet weight of granulation tissue has significantly increased compared to the non-diabetic normal levels in the essential oil-treated groups. The granulation tissue weight for both the aqueous and oil-treated diabetic groups has significantly increased compared to the untreated control groups on days 6, 10 and 15 (Table 3).

3.4. Wound contraction

Quantitative measurements of wound size are routinely used to assess initial wound size before and after debridement, as well as progress toward wound closure. The wound contraction rate was measured as the percentage reduction in wound size on days 3, 6, 10 and 15 after wounding. Significant progress in the percentage of wound contraction was observed in the treated excision wounds compared with the untreated controls (Table 4). On day 3, a sig-

Table 2
Effect of *Rosmarinus officinalis* aqueous extract and essential oil on body weight of alloxan-diabetic mice for 15 days after cutaneous full-thickness excision wound creation.

Group	Day after wounding				
	0	3	6	10	15
	Body weight (g)				
I	20.87 ± 0.26	20.77 ± 0.20	21.03 ± 0.15	20.95 ± 0.11	21.97 ± 0.13
II	19.70 ± 0.30*	18.22 ± 0.24*	17.00 ± 0.24*	16.17 ± 0.09*	14.70 ± 0.16*
III	19.38 ± 0.28*	17.95 ± 0.26*	17.93 ± 0.18*	18.58 ± 0.22**	19.73 ± 0.18**
IV	19.47 ± 0.28*	18.38 ± 0.18*	18.73 ± 0.19**	19.77 ± 0.20**	20.42 ± 0.28**

Data are expressed as means ± SEM for six mice in each group. I: non-diabetic control; II: diabetic control; III: aqueous extract-treated diabetic group (I.P. injection of 0.2 ml at a dose of 10% for 3 days); IV: essential oil-treated diabetic group (T.A. of 25 μl/excision wound, twice a day for 3 days).
* Statistically significant when compared to non-diabetic control group (I) at $p < 0.01$.
** Statistically significant when compared to diabetic control (II) at $p < 0.01$.

Table 3
Effect of *Rosmarinus officinalis* aqueous extract and essential oil on the weight of granulation tissue formed at various days after cutaneous wounding in alloxan-diabetic mice.

Group	Day after wounding			
	3	6	10	15
	Granulation tissue weight (mg)			
I	2.26 ± 0.11	2.75 ± 0.15	3.90 ± 0.16	5.43 ± 0.16
II	1.11 ± 0.13*	2.00 ± 0.13*	2.50 ± 0.17*	3.55 ± 0.19*
III	1.61 ± 0.13*	2.90 ± 0.14**	4.23 ± 0.18**	5.86 ± 0.23**
IV	2.23 ± 0.16**	3.30 ± 0.19**	4.55 ± 0.14**	6.11 ± 0.22**

Data are expressed as mean ± SEM for six excision wounds in each group. I: non-diabetic control; II: diabetic control; III: aqueous extract-treated diabetic group (I.P. injection of 0.2 ml at a dose of 10% for 3 days); IV: essential oil-treated diabetic group (T.A. of 25 μl/excision wound, twice a day for 3 days).
* Statistically significant when compared to non-diabetic control group (I) at $p < 0.01$.
** Statistically significant when compared to diabetic control (II) at $p < 0.01$.

nificant increase toward non-diabetic normal level was detected in essential oil-treated group. On days 6, 10, and 15, both the aqueous extract and essential oil had significantly increase in the percentage of wound contraction more than that observed in non-diabetic mice compared to the untreated diabetic group (Table 4).

3.5. Histological evaluation

Enhancement of the wound healing process was observed on days 6 and 15 after wounding in the treated groups compared with those of the untreated diabetic and non-diabetic controls (Figs. 1–3). On day 6, in complete full-thickness re-epithelialization besides immature, thick and disorganized epidermis with debridement crust overlying the area of the wound was observed in all groups compared to untreated diabetic wounds, which showed early full-thickness re-epithelialization. The aqueous extract was found to have more advanced degree of epithelialization and basement membrane establishment than essential oil-treated wounds as well as untreated non-diabetic wounds. Highly cellular granulation tissue with newly formed and deposited extracellular matrix elements, prominent wide capillary-sized blood vessels, invasion of inflammatory cells consisting mainly of macrophages and few neutrophils were present in the dermis of all groups except for untreated diabetic wounds, which showed delayed coagulation and inflammation. The poorly formed granulation tissue of untreated diabetic wounds was loaded with red blood cells and neutrophils along with scarce fibroblasts, macrophages and deposition of extracellular matrix elements. More advancement in the regeneration of dermal architecture, revealing large amounts of deposited extracellular matrix elements and narrow capillary-sized blood vessels were detected in the essential oil-treated wounds as compared with the extract and untreated non-diabetic wounds (Fig. 1). On day 15, a well-advanced in the maturation of granulation tissue in the dermis and on-going epithelialization was observed in treated and in non-diabetic normal wounds (Fig. 2). These progressive changes in the epidermal and dermal architecture include: keratinization and full-thickness epidermal regeneration, which covered largely the wound area, intense improvement in the maturation and organization of epidermal layers with no debridement crust covering the epidermal surface, an increase in deposition and organization of extracellular matrix elements and the presence of highly vascularized areas in the granulation tissue that were associated with several empty vacuoles, specifically at the wound site of the mice treated with the aqueous extract or essential oil. In contrast, immature and disorganized epidermal layers due to delay in full-thickness re-epithelialization, fewer and less obvious areas of blood vessels were noted throughout the scantly formed granulation tissue of the untreated diabetic mice. This further characterized by few fibroblasts, less dense collagen fibers that appeared haphazardly-oriented, as detected by Masson trichrome stain, and persistence of inflammatory cells including neutrophils (Fig. 3). Whereas, more distinct, thick, densely associated and well-organized collagen fibers/bands with normal fibroblasts alignment and persistence of inflammatory cells were found in late granulation tissue of the mice that had topical application of essential oil, when compared to extract-treated and non-diabetic normal mice, respectively. The later had distinct, less thick, coarse and randomly arranged collagen fibers appeared throughout the granulation tissue.

Table 4
Effect of *Rosmarinus officinalis* aqueous extract and essential oil on the percentage of excision wound contraction at various days after cutaneous wounding in alloxan-diabetic mice.

Group	Day after wounding			
	3	6	10	15
	Percentage of wound contraction			
I	22.66 ± 0.80	38.13 ± 1.04	71.83 ± 1.70	84.83 ± 1.24
II	11.58 ± 0.76*	21.00 ± 1.15*	45.50 ± 1.68*	64.25 ± 1.41*
III	15.33 ± 0.80*	40.25 ± 1.70**	75.91 ± 1.84**	88.83 ± 1.24**
IV	22.50 ± 1.38**	45.25 ± 1.40**	78.33 ± 1.83**	92.91 ± 1.06**

Data are expressed as mean ± SEM for six mice in each group. I: non-diabetic control; II: diabetic control; III: aqueous extract-treated diabetic group (I.P. injection of 0.2 ml at a dose of 10% for 3 days); IV: essential oil-treated diabetic group (T.A. of 25 μl/excision wound, twice a day for 3 days).
* Statistically significant when compared to non-diabetic control group (I) at $p < 0.01$.
** Statistically significant when compared to diabetic control (II) at $p < 0.01$.

4. Discussion

The possible mechanism of hypoglycemic effect observed in both aqueous extract- and essential oil-treated diabetic mice (Table 1), may be attributed to compound(s) responsible for the antioxidant effect that protect beta cells from further damage by alloxan and facilitate their regeneration leading to enhancement in

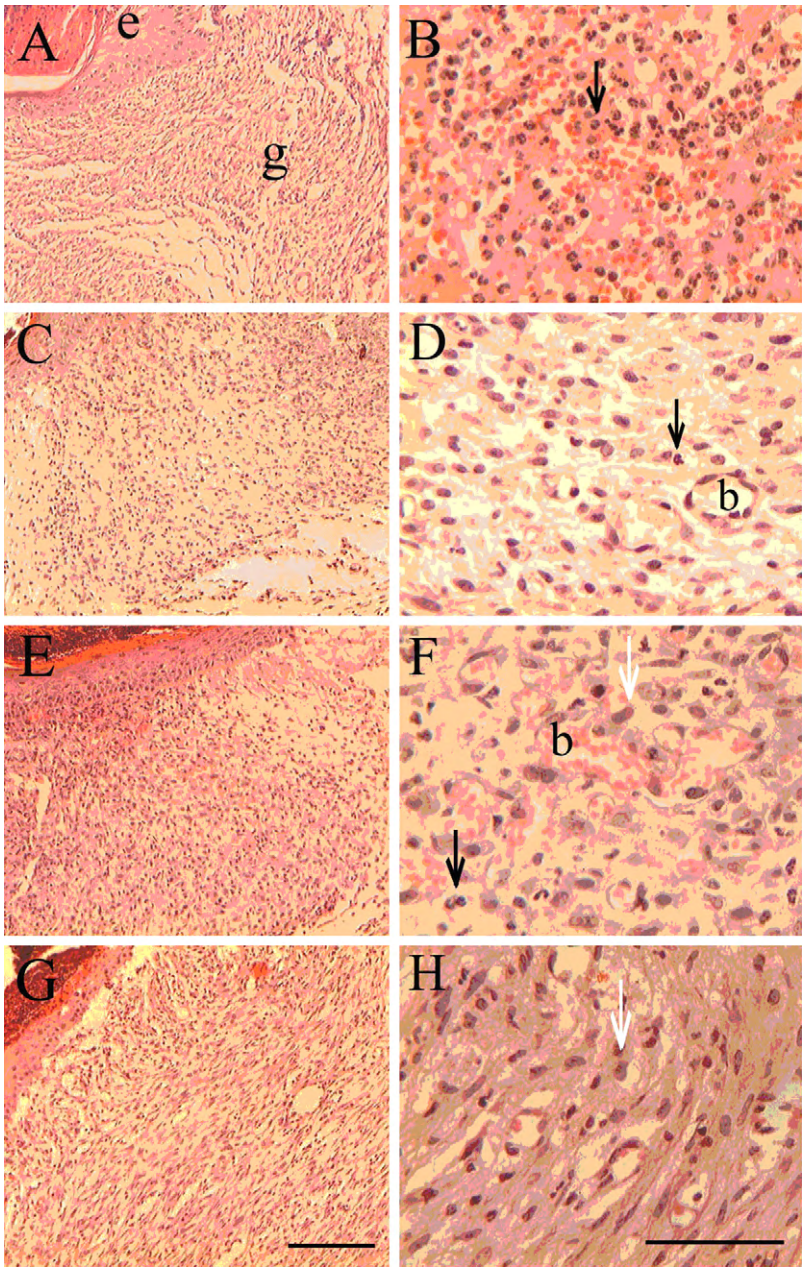

Fig. 1. Hematoxylin and eosin staining histological sections of cutaneous wound site obtained from the controls and *Rosmarinus officinalis* treated BALB/c mice on day 6 after wounding. (A and B) Untreated diabetic wound; (C and D) untreated normal wound (non-diabetic); (E and F) diabetic wound treated with *Rosmarinus officinalis* aqueous extract; (G and H) diabetic wound treated with *Rosmarinus officinalis* essential oil. Thicken and immature epidermis (e) with debridement crust overlying the area of the wound. Prominent and dilated blood vessels (b); immature granulation tissue area (g) showing invasion of inflammatory cells (neutrophill: black arrow head; macrophage: white arrow head). Scale bars: 50 μm (A, C, E, and G); 200 μm (B, D, F, and H).

insulin secretion. This later may recover the disorders in carbohydrate metabolism and/or intracellular glucose utilization, caused an improvement in body weight gained nearly to normal in the treated mice compared with the untreated diabetic mice (Table 2) (Tsuneki et al., 2004; Mallick et al., 2006; Zhang et al., 2009). Previous studies confirmed the presence of powerful antioxidant agents in crude extracts and essential oil of the aerial part of the herb such as carnosol, rosmanol, epirosmanol, carnosic acid, borneol, verbenone and rosmarinic acid (Calabrese et al., 2000; Angioni et al., 2004; Ramírez et al., 2004; Moreno et al., 2006). The relationship of antioxidant potential of the herb and its hypoglycemic effect has been reported. The ethanol extracts of *Rosmarinus officinalis* leaves significantly lower blood glucose level and increase serum insulin concentration in alloxan-induced diabetic rabbits, suggesting a capability of the extract to inhibit lipid peroxidation and activate antioxidant enzymes (Bakirel et al., 2008).

Wound healing is a complex natural regeneration process of skin cells to minimize or eliminate scarring as well to help heal and repair damage. The normal subsequent events of cutaneous healing occur in three overlapping phases: coagulation and inflammation, proliferation and remodeling (Eming et al., 2007). Coagulation and inflammation begin immediately after injury and are characterized by platelet aggregation to control excessive blood loss from the damaged vessels and the influx of inflammatory cells into the wound site. These cells play multiple roles in wound healing, including release of protease for wound debridement, phagocytosis of debris and bacteria, and secretion of various cytokines and growth factors. Which, in turn, cause migration and division of cells

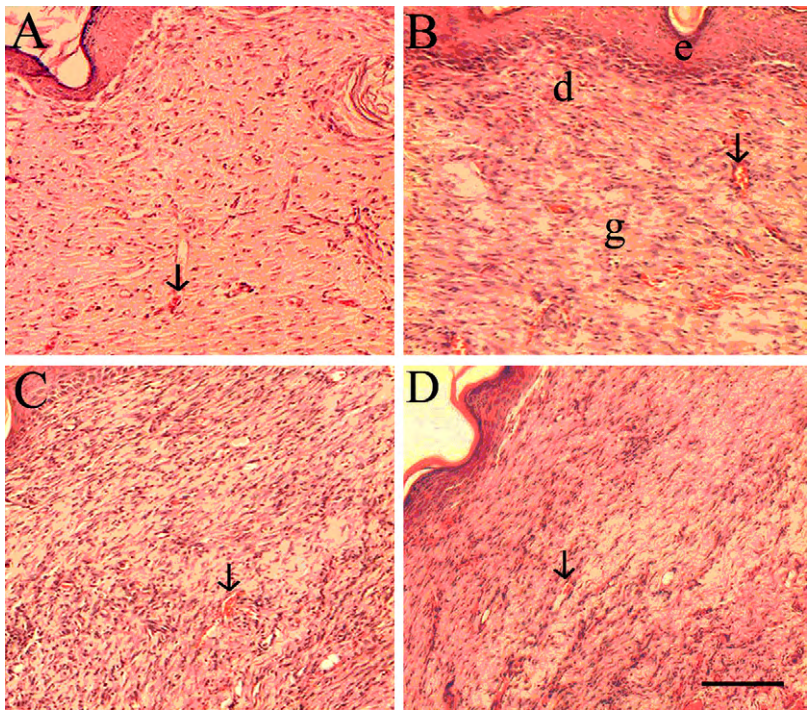

Fig. 2. Hematoxylin and eosin staining histological sections of cutaneous wound site obtained from the controls and *Rosmarinus officinalis* treated BALB/c mice, revealing epidermal and dermal architecture of the diabetic wounds on day 15 after wounding. (A) Untreated diabetic wound; (B) untreated normal wound (non-diabetic); (C) diabetic wound treated with *Rosmarinus officinalis* aqueous extract; (D) diabetic wound treated with *Rosmarinus officinalis* essential oil. Epidermis (e); dermis (d); granulation tissue (g); blood vessels (black arrow head). Scale bar: 50 μm (A–D).

involved in the proliferative phase (Stipcevic et al., 2006). During this phase, angiogenesis, collagen deposition, epithelialization and newly formed granulation tissue, consisting of endothelial cells, macrophages, fibroblasts and the components of a new provisional extracellular matrix begin to cover and fill the wound area to restore tissue integrity (Midwood et al., 2004; Eming et al., 2007). However, the remodeling phase of repair involves collagen cross linking and reorganization, evolution of granulation tissue into scar tissue and cells no longer needed are removed by apoptosis (Rai et al., 2005).

Rosmarinus officinalis aqueous extract and essential oil accelerate the wound healing in diabetic BALB/c mice by influencing different aspects of the healing process at various days after wounding, under the conditions of the present investigation (Tables 3 and 4 and Figs. 1–3). However, the healing effect of essential oil was more effective than that observed with the extract.

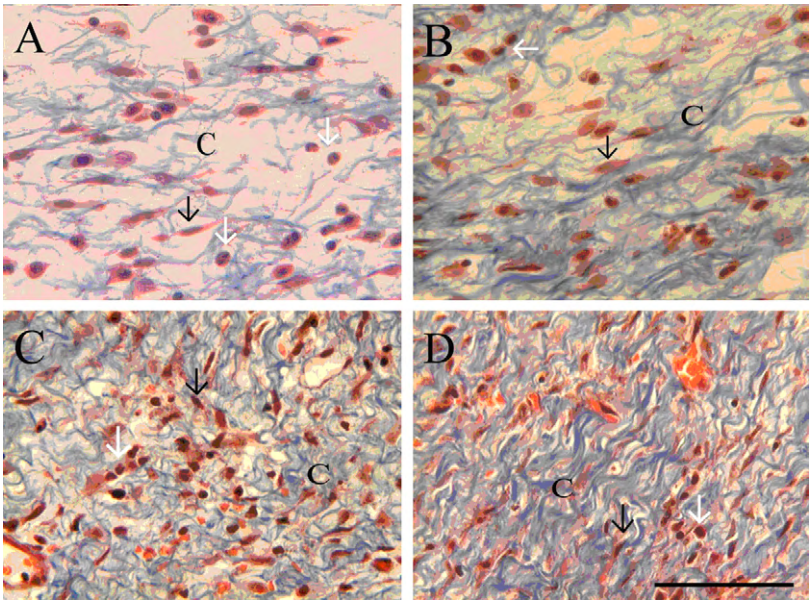

Fig. 3. Masson trichrome staining histological sections of cutaneous wound site obtained from the controls and *Rosmarinus officinalis* treated BALB/c mice, revealing dermal architecture of late granulation tissue on day 15 after wounding. (A) Untreated diabetic wound; (B) untreated normal wound (non-diabetic); (C) diabetic wound treated with *Rosmarinus officinalis* aqueous extract; (D) diabetic wound treated with *Rosmarinus officinalis* essential oil. Deposition and arrangement of collagen fibers/bands (c); fibroblasts (black arrow head); inflammatory cells (white arrow head). Scale bar: 200 μm (A–D).

Potential healing of *Rosmarinus officinalis* was high-lighted by reduced inflammation and wound debridement (Fig. 1), closure of the wound area due to rapid wound contraction (Table 4), full-thickness epidermal regeneration and organization (Fig. 2), increase in the wet weight of granulation tissue indicating rapid maturation due to high vascularization and deposition of extracellular matrix elements involving collagen and fibroblasts (Table 3 and Figs. 2 and 3). Furthermore, histological findings revealed normal cutaneous architecture of the treated wounds more than that appeared in the untreated non-diabetic wounds. The healing capability of *Rosmarinus officinalis* was evident when compared to the impaired healing process of the wounds administered to the untreated diabetic mice that showed a delay in coagulation and inflammation phase of healing on day 6 after wounding (Fig. 1). This could be associated with an increase in the number of acute inflammatory cells, impaired leukocytes function, the metabolic abnormalities of diabetes, inadequate migration of neutrophils and macrophages to the wound along with reduced chemotaxis, failure in the resolution of inflammation and a defect in neutrophil apoptosis (Gutiérrez-Fernández et al., 2007; Hirsch et al., 2008). In addition, reduced microcirculation and diminished expression of growth factors contribute to the disruption of wound healing in diabetes, such as impairment in wound closure and/or complete epithelialization (Table 4 and Fig. 2), fibroblasts proliferation leading to inadequate deposition of fibrous collagen tissue (Fig. 3) and scarcely formed granulation tissue (Table 3 and Fig. 3) (Sivan-Loukianova et al., 2003; Lerman et al., 2003; Cianfarani et al., 2006; Eming et al., 2007; Silva et al., 2007).

Healing potential of *Rosmarinus officinalis* could be explained on the basis of the powerful anti-microbial (Angioni et al., 2004; Oluwatnyi et al., 2004), anti-inflammatory (Takaki et al., 2008) and antioxidant (Calabrese et al., 2000; Moreno et al., 2006) effects of the herb that are well documented in the literature. It has been reported that if a wound becomes infected, the normal healing is disrupted as the inflammatory phase becomes chronic suppressing the proliferation phase of healing (Yates et al., 2007). The acute inflammatory during the early stages of wounding generates factors that are essential for tissue growth and repair. When prolonged, as the case in diabetes, preventing wound remodeling and matrix synthesis, leading to delay in wound closure (Eming et al., 2007; Gutiérrez-Fernández et al., 2007). The production of free radicals at or around the wound bed may contribute to delays in wound healing through the destruction of lipids, proteins and extracellular matrix elements (Calabrese et al., 2000). Due to these properties, the herb may facilitate wound healing by reducing local inflammation and tissue destruction, increasing angiogenesis and collagen deposition leading to improvements in both local circulation and granulation tissue formation.

The main bioactive compounds isolated from both aqueous and organic extracts of the aerial parts of Rosemary having potential effect on inhibiting pathogenic growth, reducing inflammatory response and preserving viable tissue are mostly terpenoids and polyphenols such as carnosol, carnosic acid and rosmarinic acid (Calabrese et al., 2000; Oluwatnyi et al., 2004; Moreno et al., 2006; Takaki et al., 2008). The quality and chemical composition of the oil depend on how and where the plant was grown, harvested, and distilled (Angioni et al., 2004; De Mastro et al., 2004; Sotomayor et al., 2009). When conditions cause the plants to permanently produce variations in the chemical composition of their essential oils, these plants are known as chemotypes. The main constituents are α- and β-pinene, 1, 8-cineole, camphor, verbenone, borneol and limonene. Often all these chemicals can exist in the oil, however there are principal chemotypes (CT) of *Rosmarinus officinalis*, with the names given by one of the main constituents, such as α-pinene, camphor/borneol, cineole, and verbenone CT (Pintore et al., 2001; Lahlou and Berrada, 2003). Each one of the Rosemary species chemotypes has a different essential oil composition and hence, a different range of therapeutic benefits (Celiktas et al., 2005; Sotomayor et al., 2009). In this study, a representative sample of *Rosmarinus officinalis* was harvested from different locations in Al-al-Bayt University campus due to differences in the quality of the soil, fertilizers and irrigation that may represent at least one or more chemotypes. Therefore, further studies should be followed in the future to confirm and assess the range of wound healing potential of essential oils of Rosemary chemotypes collected from different locations, at several time intervals, in Jordan.

5. Conclusions

Essential oil from the aerial parts of *Rosmarinus officinalis* exhibited superior significant healing effect over the aqueous extract, when topically applied on the wound of diabetic mice, by affecting various stages of the healing process. The result of the present study offers scientific pharmacological evidence on the folkloric use of *Rosmarinus officinalis* aerial parts for healing wounds. However, further scientific verification is required to confirm and assess the range of wound healing potential of essential oils of Rosemary chemotypes collected from different locations, at several time intervals, in Jordan.

Conflict of interest

The author has declared that no conflict of interest exists.

Acknowledgments

The author acknowledge Wasfi Al-bekearat for his support and technical assistance, Suhail El-Suhaile, who kindly prepared the essential oil of *Rosmarinus officinalis*, Al-al-Bayt University, Department of Biological Sciences, Mafraq, Jordan, Ismail Zaid for the help in photographing histological sections, Yarmouk University, Department of Biological Sciences, Irbid, Jordan and Al-al-Bayt University, Mafraq, Jordan, for providing necessary facilities to carry out the study.

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